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PELDRI II: A Quick and Easy Alternative to Critical Point Drying for Scanning Electron Microscopy

BRIGITTE ZIMMER

Botanischer Garten und Botanisches Museum Berlin-Dahlem, Königin-Luise-Str. 6–8, D-1000 Berlin 33, Germany

Micromorphological data are increasingly used in pteridophyte taxonomy for improving the classification and the circumscription of taxa. Recent examples, among many others, are Barrington (1978, p. 5) who considers the indumentum (epidermal structures) of the petiole as crucial for the classification of *Trichipteris*, and Hovenkamp (1986, p. 54) who found that some *Pyrrosia* species which are similar in other respects may be distinguished by the shape of epidermal cells.

At present, most scanning electron microcopic (SEM) studies in fern taxonomy concentrate on the investigation of spores (e.g., Tryon & Tryon, 1982). This may be partly explained by the fact that fern spores need no special pretreatment such as fixation or drying for SEM examination and that therefore comparative studies can be performed quickly and easily, and at low cost.

The SEM investigation of soft structures and tissues, including most epidermal features in ferns where cutinisation and sclerification of cell walls is low, gives unsatisfactory results when air-dried samples are used. The critical point drying (CPD) technique, introduced long ago by Anderson (1951) and nowadays routinely used in SEM studies, is adequate in such cases. CPD preparation however requires special equipment. The number of different samples that can be treated simultaneously is dependent on their size and on the equipment used. This is often a limiting factor and a serious handicap for the application of CPD in taxonomic research, where the comparative study of a large number of samples is essential.

The chemical PELDRI II has been reported to give good results in drying animal tissues for SEM by sublimation (Kennedy et al., 1989) and has the advantage of allowing the simultaneous treatment of a large number of samples, without special equipment and in a comparatively short time. The question of whether it can provide a good alternative to CPD for the study of micromorphological characters in ferns has therefore been investigated.

MATERIALS AND METHODS

Pinnae of fern fronds of various genera and species, cultivated in greenhouses at the Berlin Botanical Garden were pressed and air-dried as for the preparation of normal herbarium specimens. For CP-drying or treatment with PELDRI II, other pinnae of the same fronds were fixed in FAA (90 ml ethanol (70%), 5 ml acetic acid (100%), 5 ml formaldehyde (> 37%)). After 24 hours of FAA-fixation the fixing solution was completely changed and the probes were cut into pieces. After another 24 hours they were dehydrated through a graded series of ethanol

(70%, 80%, 90%, 96%, 100%, 100%; 1 hour in each), and the ethanol was finally replaced by acetone (100%, 100%). The fixation and dehydration were carried out at room temperature.

For the CP-drying with liquid CO_2 (with 9 complete changes of CO_2 to substitute the acetone completely) a critical point drying apparatus (Polaron E 3000) was used.

The procedure with PELDRI II (W. Plannet GmbH, PELCO INTERNATIONAL), performed under a fume hood, was as follows: PELDRI II, which is solid at room temperature (melting point 23.8°C), was liquified on a hot plate (28–30°C). The vessels containing the fixed material in 100% acetone were placed alongside the PELDRI II vessels on the hot plate, and an equivalent amount of PELDRI II was added to each of them to give a 1 : 1 mixture (using pre-heated pipettes to avoid re-solidification). After at least 1 hour, when the samples had sunk to the bottom of the vessels, the mixture was removed and replaced by 100% PELDRI II (if the samples were unusually slow to sink, the exchange time was prolonged and/or a second exchange was performed). For final sublimation PELDRI II was allowed to re-solidify by cooling to room temperature after removal from the hot plate, after which the vessels were transferred to a vacuum desiccator connected to a water-operated pump of a current type and placed under vacuum (overnight) until the sublimation of PELDRI II was completed. The time of sublimation will vary depending on the amount of the chemical, on the surface area (width of the vessels used), and on the vacuum pressure achieved. (Users' instructions accompanying PELDRI II recommend a vacuum of 5×10^{-2} mbar or less, as can be generated by a mechanical pump; however, the less sophisticated equipment used by us yields entirely satisfactory results.) The glass vessels were kept uncapped throughout preparation.

CP-, PELDRI II-, and air-dried samples were mounted on stubs, coated with gold (thickness c. 15 nm) by a sputter-coater (Technics, Hummer I) and examined by a scanning electron microscope (I.S.I., Super III A) at 15 kV. For photographic documentation a Mamiya 6×7 camera and Ilford FP4 120 film were used.

Results and Discussion

The aspect of the controls confirmed that air-dried material is of little use for surface morphological investigations by SEM (Fig. 1 a, b; Fig. 2 a, b), especially in taxa in which the cuticular outer layer is weak (Fig. 2).

The results of PELDRI II treatment with this tissue were fully comparable to those achieved by CPD. In general, the surface morphology of the leaves is well preserved and structural features are clearly visible. No difference in quality is apparent between PELDRI II treated and CP-dried leaf surfaces of, e.g., *Adiantum caudatum* L. (Fig. 1 c, e) or *Saccoloma inaequale* (Kunze) Mett. (Fig. 1 e, f). The shapes and patterns of epidermal cells and stomata can be equally well demonstrated with both methods. Trichome features are less satisfactory, the trichomes being partly collapsed in both preparations (Fig. 1 d, f).

Excellent results were also obtained with the delicate leaves of filmy ferns,

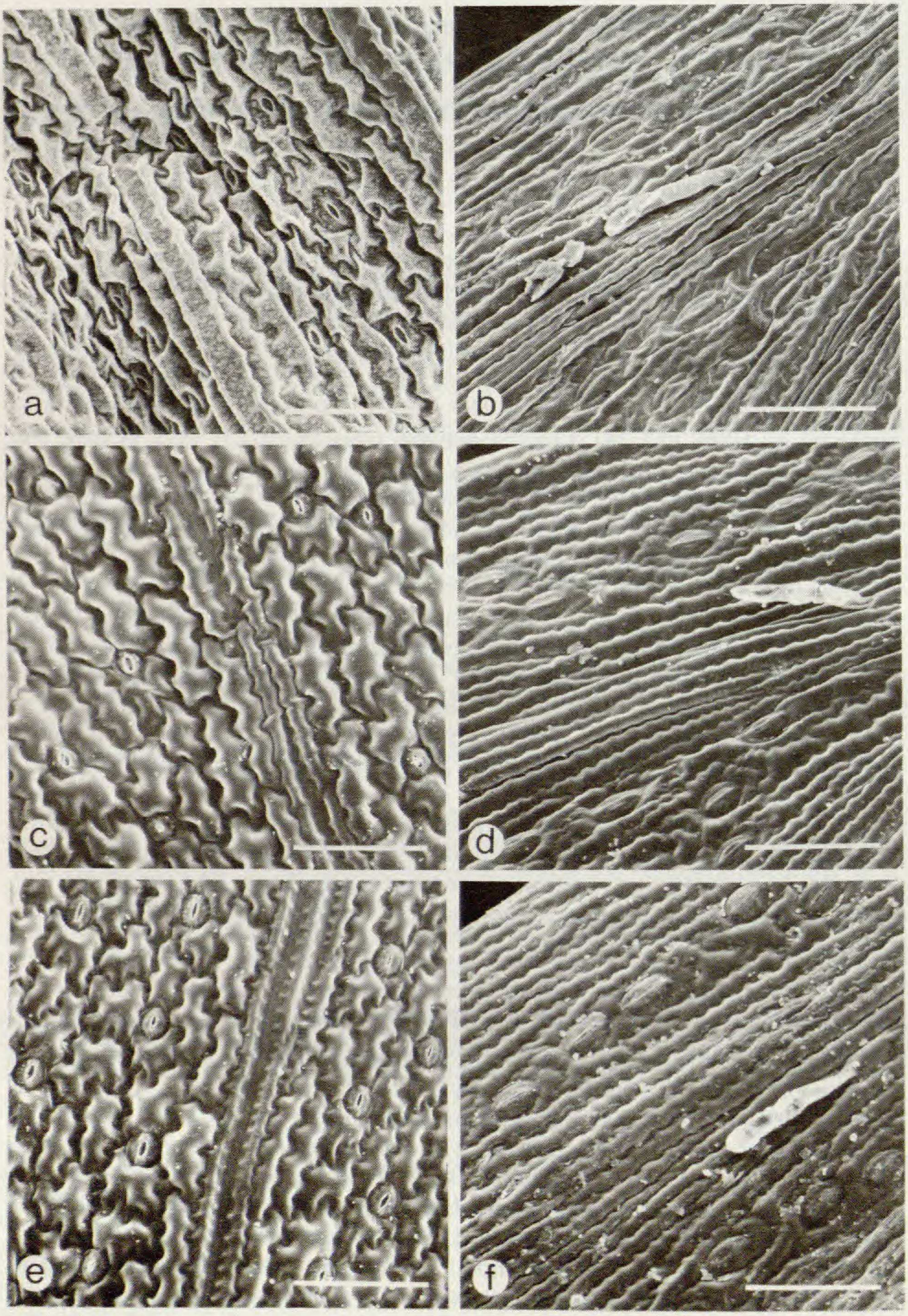

FIG. 1. SEM photographs of lower leaf surfaces of *Adiantum caudatum* (a, c, e) and *Saccoloma inaequale* (b, d, f). Specimens air-dried (a, b), PELDRI II-treated (c, d), and CP-dried (e, f). Bar = 300 μm.

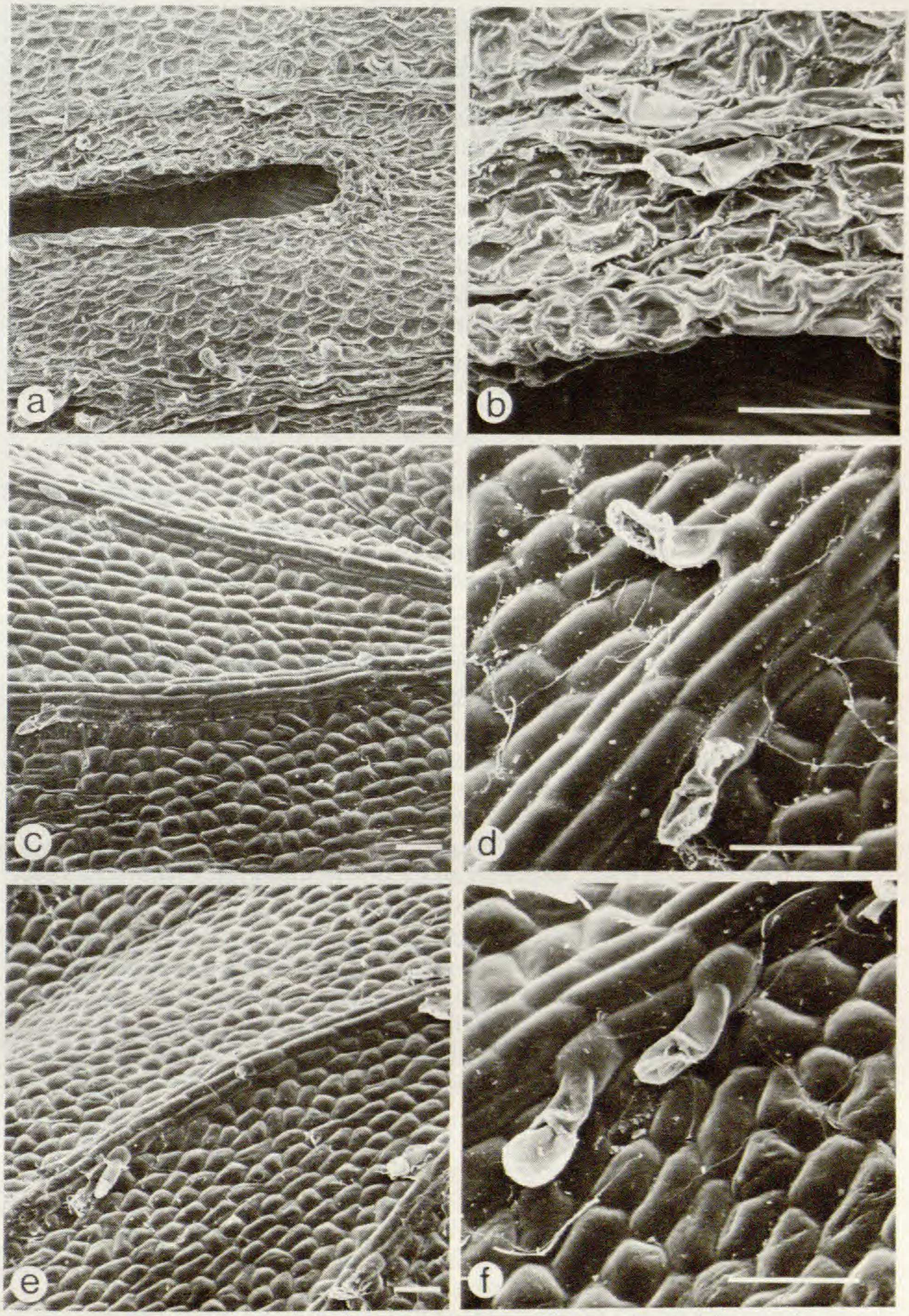

FIG. 2. SEM photographs of upper leaf surfaces of *Trichomanes radicans*. Specimens air-dried (a, b), PELDRI II treated (c, d), and CP-dried (e, f). Bar = 300 μm.

again with both techniques, as exemplified by *Trichomanes radicans* Sw. (Fig. 2 c, d, e, f). Here again the epidermal cells, this time of the upper leaf surface, and the bases of trichomes have a completely natural appearance, whereas the apical parts of the trichomes show signs of partial collapse.

The quality of preservation of fern leaf tissues dried by sublimation with PELDRI II is equal to that of critical-point-dried material. The partial collapse of epidermal trichomes in PELDRI II-treated as well as in CP-dried preparations probably takes place prior to fixation. The plant material was cut in the greenhouse and immediately fixed, but the delicate trichomes may well have had partially lost their turgescence beforehand.

The advantage of PELDRI II treatment for systematic research is obvious, since no expensive apparatus is needed and a great number of samples can be treated simultaneously under equal conditions, requiring a relatively modest amount of time and labor.

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